

REMARKS

Claims 1-28 are active in the present application. Favorable consideration is respectfully requested.

Applicants submit that this application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,  
MAIER & NEUSTADT, P.C.



Norman F. Oblon  
Attorney of Record  
Registration No. 24,618

James J. Kelly, Ph.D.  
Registration No. 41,504



**22850**

(703) 413-3000  
Fax #: (703) 413-2220  
NFO/JK/TWB:cja

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Herewith:**RECEIVED**

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**TC 1700**IN THE SPECIFICATION

Page 4, please replace the paragraph at lines 11-20 as follows:

--In addition, the qualities of oxygen infusions deteriorate with time due to the inherent characteristics of heme protein, and therefore it is difficult to preserve them in a stable condition. More specifically, hemoglobin, lipid heme and heme derivatives can reversibly bond with oxygen when the central iron of heme is a ferrous iron ( $\text{Fe}^{2+}$ ), whereas when the ferrous iron is oxidized to a ferric iron ( $\text{Fe}^{3+}$ ), oxygen binding capability is lost. Further, even a ferrous complex bound with oxygen is gradually oxidized automatically while releasing superoxide anion  $[(\text{O}_2^-)]$  ( $\text{O}_2^-$ ), and is finally converted to a ferric iron. Thus, the complex loses its oxygen binding capability (for example, hemoglobin becomes methemoglobin). Further, heme protein thus converted to a met-form can easily release free heme and free ferric iron, which is a concern causing adverse effects on the living body.--

Page 14, please replace the paragraph at lines 12-26 as follows:

--(7) About 0.2 mL of each sample was lyophilized and the membrane components were extracted with about 1 mL of  $\text{CDC1}_3$ , followed by filtration with a filter. Then, the resultant sample was measured in terms of  $[1\text{H}]^1\text{H-NMR}$  spectrum (JNM-LA500, Nihon Denshi). On the other hand, in order to remove polyoxyethylene chains dissociated external aqueous phase, about 0.2 mL of each sample was diluted with PBS by ab

folds and the supernatant liquid was removed by an ultracentrifugal separation (100,000g, 15 min). After the sediments was re-suspended with PBS, the resultant was freeze-dried, and then the membrane components were extracted using about 1 mL of  $\text{CDCl}_3$ , followed by a filtration with a filter. Then, the resultant was measured in terms of  $^1\text{H}$ -NMR spectrum. The peak (B) which is assigned to the methylene protons of polyoxyethylene chain in polyoxyethylene lipid appeared at  $\delta$  : 3.63 ppm, whereas the peak (A) which is assigned to choline methyl proton of phosphatidylcholine appeared at  $\delta$  : 3.39 ppm. Supposing that the ratio between the number of protons in the peak (A) to that in the peak (B) is equal to the integral ratio of B/A, the incorporation ratio of polyoxyethylene chains was calculated by way of the following formula:--

Page 14, please replace line 29 as follows:

--B/A(after) is the Integral ratio B/A after removal of external water phase[]]; and--

Page 15, please replace the paragraph beginning at line 30 to page 16, line 10 as follows:

--The obtained oxygen infusion [according to the present invention] was subjected to preservation test. Preservation conditions used entailed preservation in a refrigerator (4°C), room temperature preservation (23 °C), and preservation in an incubator (40°C). The following measurements were carried out for the samples for six months and the measured results were compared with the sample before these preservations. That is, the presence/absence of a sediment formation in the sample in each case was visually observed with naked eyes. 30 mL of each sample was diluted by 10 folds with physiological saline, and then, subjected to measurement in terms of absorbance at 900 nm using a 1-mm cuvette at room temperature. The absorbance of the physiological saline at 900 nm was subtracted as

a reference from the measured value, and thus obtained value was taken as the turbidity of the respective sample. The measurement of the distribution of particle diameters was conducted by a dynamic light-scattering method using Sub-micron Particle Analyzer Model N4 SD (Coulter Corporate Communications) at a temperature of 25°C.--

Page 17, please replace the paragraph beginning at line 19 to page 18, line 4 as follows:

--A lipid heme vesicle suspension was made of 5, 10, 15, 20-tetrakis [ $\alpha, \alpha, \alpha, \alpha$ -o- {2'-dimethyl-20' (2"-trimethylammonioethyl)phosphonatoxyeicosanamido}-phenyl]porphyrinato-iron(II) (lipid heme)/1-stearylimidazole/dipalmitoyl phosphatidyl choline/cholesterol/polyoxyethylene-conjugated phospholipid which is -- (monomethoxypolyoxyethylenecarbamyl) diphosphatidyl ethanolamine, in a molar ratio of 1/3/40/20/2.5. The average molecular weight of the polyoxyethylene chains was adjusted to 5000. To the suspension, physiological saline was added to prepare a solution having a lipid heme concentration of 5 mM. The solution was subjected to the extrusion method described in Example 1 so as to control the particle diameter, and then sealed into a glass container with addition of 6 mM of ascorbic acid. Then, a nitrogen gas was put through the solution by the same method as in Example 1. As a result, ferric iron hemes were all reduced to ferrous iron hemes and the oxygen partial pressure was reduced to low as 3 Torr; therefore substantially all of the vesicles in the container became deoxy-type lipid heme vesicles. Thus obtained infusion was preserved at room temperature for three months, and the analysis thereof did not show any indication of increase in the amount of ferric iron heme. Further,

the particle diameter was  $105 \pm 21$  nm before the preservation, whereas after the preservation, it was  $107 \pm 28$  nm, exhibiting no substantial change. A significant increase in turbidity was not observed.--

Page 18, please replace the paragraph at lines 6-23 as follows:

--For the preparation of a lipid heme - triglyceride microsphere suspension, a soybean oil ([soybean oil]/[heme] = a ratio of 2 to 4 by weight) was added to 5, 10, 15, 20-tetrakis [ $\alpha$ ,  $\alpha$ ,  $\alpha$  -o-{2', 2' -dimethyl-20' (2''trimethylammohioethyl)phosphonatoxyeicosanamido}-phenyl] porphyrinato-iron(II) (lipid heme)/1-stearylimidazole (at a ratio in molar of 1/2.5), and further a 2%-glyceline aqueous solution was added thereto. Then, the mixture was subjected to supersonic agitation in a water bath under a nitrogen atmosphere, thus obtaining the suspension. To the suspension, a polyoxyethylene-conjugated lipid having an average molecular weight of 2000, N-(monomethoxypolyoxyethylenecarbamyl) dipalmitoylphosphatidyl ethanolamine, was added at a ratio of 0.02 mol% with respect to the lipid heme, so as to modify the lipid heme - triglyceride microsphere with polyoxyethylene. 180 mL of thus obtained suspension was then sealed into a 200 mL-glass container with a slightly excessive amount of ascorbic acid added therein. Then, the bubbling with a nitrogen gas was performed by the same method as in Example 1, and thus the oxygen partial pressure was reduced to low as 2 Torr. Thus, deoxy-type lipid heme - triglyceride microspheres were obtained. The resultant suspension was preserved at room temperature for four months, and the analysis thereof did not show any indication of increase in the amount of ferric iron heme. Further, the particle diameter was  $85 \pm 25$  nm before the preservation, whereas after the preservation, it was  $86 \pm 28$  nm, exhibiting no substantial change.--

Page 18, please replace the paragraph beginning at line 25 to page 19, line 4 as follows: